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Anant Babu Marahatta
Department of Chemistry, GEMS Institute for higher education, GEMS School, Lalitpur, Nepal

Bharat Poudel
(1). Department of Chemistry, GEMS Institute for higher education, GEMS School, Lalitpur, Nepal
(2). Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Ram Chandra Basnyat
Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Correspondence
Anant Babu Marahatta
Department of Chemistry, GEMS Institute for higher education, GEMS School, Lalitpur, Nepal

The Phytochemical and Nutritional analysis and biological activity of *Tectaria coadunate* Linn.

Anant Babu Marahatta, Bharat Poudel and Ram Chandra Basnyat

Abstract
We selected *Tectaria coadunate* (kali niyuro, कालिन्युरो in Nepalese language), a very common local nutritious vegetable in Nepal, to study its phytochemical and nutritional behavior. We chose methanol and hexane as polar solvents and prepared respective extracts of its leaves and rhizomes by soxhlet extraction method. We unveiled alkaloids, polyphenols and tannins as the predominant phytochemicals. The percentage composition of different nutrition parameters (moisture, total ash, crude fat, protein, crude fiber, carbohydrate) are found to be 87.23, 1.40, 0.14, 1.76, 2.42, 6.97 in fresh leaves sample and 0, 11.04, 1.11, 13.88, 19.05, 54.93 in oven dried leaves sample respectively. The vitamin C content is around 10.49 mg/100 g of the oven dried leaves sample. The total phenolic and flavonoid contents of methanol extract of rhizomes are estimated as 186.61 mg/g (GAE) and 143.72 mg/g (QE) respectively. The zones of inhibition shown by methanol extracts of leaves and rhizomes for *E. coli*, *K. pneumoniae* and *S. aureus* bacteria are measured as 16, 9, 20 mm and 15, 11, 21 mm respectively in antibacterial assay. In DPPH free radical scavenging test for antioxidant activity, the IC_{50} values of hexane and methanol extracts of leaves and rhizomes are143.49, 150.80µg/mL and 76.32, 50.81µg/mL respectively. The presence of major chemical compounds: Decenediol<1,10>, Dodecanoic acid, Methyl stearate and Palmitic acid are confirmed by GC/MS analysis.

Keywords: *Tectaria coadunate* (kali niyuro, **कालिन्युरो)**, Hexane extract, Methanol extract, Proximate Nutritional Analysis, Antimicrobial, and Antioxidant

1. Introduction
Nature stands as an infinite resource for novel chemotypes and pharmacophores and the naturally available resources continue to provide an alternative to modern medicine in drug discovery. Naturally available plants in traditional medicine are the most affordable and easily accessible source of the treatment in the primary healthcare system as they contain wide array of substances that can be used as an expectorant, astringent, and to treat bronchitis. Many medicinal plants are known to possess antimicrobial, antioxidant, antidiabetic, antimalarial, anthelmintic, anticancer properties [1-3]. Plants are capable of synthesizing large varieties of organic compounds of very unique and complex structures which are categorized as primary metabolites and secondary metabolites (hereafter, SMs). The SMs like steroids, terpenoids, flavonoids, alkaloids, quinones, polyphenols etc. are biosynthesized from primary metabolites [4].
Since ancient times and up to this day, mankind has been using SMs as resources for medicines, spices, fragrances, pesticides, poisons, stimulants, dyes, perfumery and countless more purposes. The formation of certain SM compounds may be restricted to single plant species, specific plant organs, cells or even particular cell compartments. Due to many difficulties of frequent extraction but having tremendous potentialities for developing wide spectrum drugs, SMs have been important targets for bioengineering [5-8].
Of the total number of species found globally, Nepal possesses 2.80 percent plants [9]. The medical herbs databases listing for Nepal shows 1,624 species of medical and aromatic species [10]. The Himalayas are more famous for medicinal plants and have even been mentioned in the Ayurveda. Many of the herbs and plants found in the Himalayas have been integral part of traditional medicine practices of indigenous community in Nepal. Nepal's share of pteridophytes is 5.15 percent and there are 2,532 species of vascular plants represented by 1,034 genera and 199 families in its protected sites [9]. That is why, Nepal is one of the largest exporter of the medical herbs as they are very reputed in international market for their medical benefits.
Pteridophytes are a group of vascular plants that reproduce via spores and have neither seeds nor flowers. Ferns and mosses belong to this group. The plant *Tectaria coadunate* (hereafter, *T. coadunate* or TC), locally known as *kali niyuro* (कालिन्युरो)**,** as shown in Figure 1 and Figure 2,

Belongs to family Tectariaceae**,** the halberd fern family (order Polypodiales), containing 7–10 genera and about 230 species. Tectariaceae is distributed nearly worldwide but is most diverse in tropical regions such as Tropical Africa, India, China, Indochina, Taiwan, Malaysia and Nepal. Rhizome is erect, up to 2 cm in diameter with scales dark brown with paler margins, lanceolate in outline, entire, tapering to a point, up to 9 mm long. Fronds are tufted, arching, and thinly herbaceous, up to 1.8 m long, with proliferating buds on rhachis, costae and costules on the upper surface.

Since, *T. coadunate* is an edible fern with high percentage dietary fibers and most popularly used as wild vegetable due to its high nutritional value, it is expected to compose mostly of water, carbohydrates, proteins with abundant vitamin A and vitamin C. The young shoots with fiddleheads are high in minerals like iron (Fe), magnesium (Mg) and potassium (K) but low in sodium (Na). They taste somewhat similar to asparagus-okra-spinach, but their texture is slightly crunchy. They are extremely perishable and need to be cooked within a day or two after picking. In Nepal, they are mostly collected in the spring time from the woods, shady swamps, riverbanks, and damp fields. They can also be purchased from the local markets. However, local Nepal ease people think that not all types of ferns are edible. Certain ferns such as Bracken (Pteridium) are thought to be carcinogenic if their fiddleheads are consumed. The rhizomes of *kali niyuro* are used against anthelmintic activity, stomach pains, gastrointestinal disorders, eradication of worms in children from early centuries. In spite of such exceptional medicinal and nutritional values of *T. coadunate*, noconcreat research works have been reported so far concentrating on its phytochemical and nutrional analysis and biological activity. In order to accomplish this major objective, we at first extracted the plant extracts and analysed thoroughly the phenolic content, flavonoid content, antibacterial assay, antioxidant assay and antibacterial assay. Furthermore, the major chemical compounds present in the plant are also revealed by GC/MS analysis of the plant extracts. The paper is organised as follows: Materials and methods, Results and Discussions and Conclusion.

2. Materials and methods

2.1. Material

The fresh leaves and rhizomes of *T. coadunate* were collected during spring 2018 from the damp fields of hilly region (Tanahu, Province no. 4), Nepal. The taxonomic identification of the plants was done at the Central Department of Botany, Tribhuvan University, Kirtipurby judging the preserved herbariums carefully.

2.2. Method

2.2.1 Extraction

The collected fresh leaves and rhizomes were washed with tap water to remove the contaminants. The leaves were shade dried and grinded into the powder form and stored in a clean zipped plastic bag until further use. The phytochemicals present in the powdered leaves and rhizomes were extracted by percolation method using widely used Soxhlet extraction method. The powdered sample was taken in two separate thimbles of Soxhlet extractor. The round bottom flask was filled two-third with hexane and was adjusted to the extractor. Finally, the solvent was heated at around 40ºC and the extraction process was allowed to run for about an hour. After the completion of the extraction process, the solvent with extract was subjected to concentration process using the rotary evaporator at 40ºC. Thus obtained hexane extract was dried over heating source and then stored properly for further use. Similarly, the methanol extract was also obtained by the similar process by heating the solvent at around 70ºC.

2.2.2 Phytochemical analysis

The phytochemicals present in two different plant extracts were analyzed by following the protocol given by Ciulei I [7].

2.2.3 Total phenolic content

The total phenolic content in plant extract was analyzed by Folin-Ciocalteu colorimetric method based on oxidation-reduction reaction as described by Waterhouse [11]. Gallic acid is used as the standard as it is less expensive and purely available than other options.

2.2.4 Total flavonoid content

The total flavonoid content in the plant extract was determined by Aluminium chloride ($AlCl_3$) colorimetric assay [12]. Quercetin is used as the standard.

2.2.5 Antibacterial assay

Inhibition of bacterial growth was tested by using an agar well plate method and measured in the form of zone of inhibition (ZOI) as given by Dingel *et al.* [13]. The antibacterial assay was performed at Gandaki Medical College, Pokhara, Nepal.

2.2.6 Antioxidant assay

Antioxidant activity of different plant extracts was done by DPPH radical scavenging method as described by Blois (1958) [14].

2.2.7 Anti-diabetic assay

Anti-diabetic activity of plant extracts was determined from α-amylase inhibition assay [15].

2.2.8 Proximate analysis of nutritional value

The approximate analysis for the nutritional composition of *T. coadunate* was determined according to the protocol given by AOAC [16].

3. Results and Discussions

3.1 Phytochemical Assay

The micro-chemical analysis of crude extract of TC leaves and rhizomes in methanol and hexane extract depicted the presence of following phytochemicals listed in Table 1. This qualitative phytochemical assay specifies that the leaves and rhizomes contain a significant amount of steroids, alkaloid, flavonoids, phenolic, saponins, and tannin content.

Table 1. Phytochemical analysis of leaves and rhizomes. Key: (+): Present (−): Absent

S.N.	Phytochemicals	Colour	Hexane	Methanol
1.	Phenolic Compounds	Greenish blue	+	+
2.	Alkaloids	Reddish gray	−	+
3.	Carbohydrates	Violet	−	+
4.	Terpenoids	Reddish gray	+	−

5.	Steroids	Yellowish	+	+
6.	Saponins	Light Maroon	+	+
7.	Tannins	Dark Maroon	+	+
8.	Flavonoids	Orange	+	+

3.2 Total phenolic and flavonoid content

By using calibration curve and absorbance values (Figure 3) (triplicates of 1000 µg/mL), total phenolic content (TPC) of methanol and hexane extracts of leaves and rhizomes of *T. coadunate* are calculated. TPC of methanol extract for leaves and rhizomes are 149.43 and 186.61 mg per gram Gallic acid equivalent (mg/g GAE) respectively. Similarly, TPC of hexane extract of leaves and rhizomes are calculated as 23.07 and 27.67 mg per gram Gallic acid equivalent (mg/g GAE) respectively (Figure 4). Additionally, by using calibration curve and absorbance values (Figure 5) (for triplicates of 1000 µg/mL), total flavonoid content (TFC) of methanol extract of leaves and rhizomes are also calculated. TFCs of methanol and hexane extract of leaves and rhizomes are estimated as 119.62 and 143.72 mg per gram quercetin equivalent (mg/g QE) and 16.41, and 22.8 mg/g QE respectively (Figure 6).

From the results obtained from TPC and TFC analysis, it is found that flavonoids show antioxidant activity (both biological and pharmacological) because of their scavenging property or chelating process. The phenolic contents which act as antioxidant and thus reduce the risk of oxidative stress induced diseases are mostly present phytochemicals in *T. coadunate*. The difference in phenolic and flavonoid content of the extracts may be attributed to the solvent used in extraction. It is apparent that methanol extracts in comparison to the hexane extracts contain high phenolic and flavonoid compounds.

3.3Antioxidant activity

The antioxidant activity of methanol and hexane extracts of *T. coadunate* are studied by plotting% free radical scavenging vs concentration (Figure 7 and Figure 8) and the half maximal inhibitory concentration (IC_{50}) values of respective extracts are calculated. Thus calculated IC_{50} value of different extracts are compared with IC_{50} value of standard solution: ascorbic acid. The IC_{50} value of ascorbic acid is calculated to be 40 µg/mL. The IC_{50} values of methanol and hexane extracts of leaves are found as 76.32 and 143.49 µg/mL and those of the rhizomes are 50.81 and 150.8 µg/mL respectively for their antioxidant activity (Figure 9). IC_{50} value of methanol extract of TC rhizomes and leaves are found to be slightly higher than that of ascorbic acid. Among the four extracts, methanol extract of TC rhizomes shows highest antioxidant activity. Similarly, methanol extract of TC leaves also show significant value for their antioxidant properties. The hexane extract of leaves and rhizome show ever, deviates strongly from ascorbic acid indicating their negligible antioxidant activity.

3.4 Anti-diabetic activity

The anti-diabetic activity of methanol extract of TC leaves and rhizomes are measured *in vitro* by taking acarbose (available anti-diabetic drug) as a standard drug and IC_{50} values are also calculated. The comparison between α–amylase inhibition activity of different extracts and acarbose is shown in Figure 10. From the study of anti-diabetic activity, IC_{50} value of the methanol extract of leaves and rhizomes are found as 326.96µg/mL and 164.76µg/mL respectively, as shown in Figure 11. These values are extremely higher than the IC_{50} value of standard acarbose (52.77µg/mL) but the values are significant so as to show considerable amount of enzyme inhibition activity (anti-diabetic activity). Comparatively, the methanol extract of TC leaves shows very low anti-diabetic activity.

3.5 Antimicrobial assay

Pathogenic bacteria cause diseases to plants, animals and mostly human beings. Dysentery, tuberculosis, respiratory infections etc. are the most common diseases caused by pathogenic microorganisms. Antimicrobial agents inhibit or kill the growth of such microorganisms. The antimicrobial activity of the plant can be evaluated by calculating several parameters such as zone of inhibition (ZOI), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The area around the antimicrobial disk where there is no growth of micro-organisms takes place is called ZOI, the minimum concentration of the plant extract that hinders the growth of microorganisms is called MIC and the minimum concentration of the plant extract that kills the microorganisms completely is called MBC. The ZOI, MIC and MBC values for the different bacterial species in methanol extracts of rhizomes and leaves are tabulated (Table 2 and Table 3) below:

Table 2: Antimicrobial activity of methanol extract of *Tectaria coadunate* rhizomes

S.N.	Bacteria	Reference culture	ZOI Value (mm)			MIC $mg.mL^{-1}$	MBC $mg.mL^{-1}$
			Positive control Ampicillin	Negative Control Methanol	TC rhizome extract		
1.	*Escherichia Coli*	ATCC 8739	27	0	15	10^{-3}	1
2.	*Klebsiella pneumonia*	ATCC 700603	18	0	11	10^{-2}	10
3.	*Staphylococcus aureus*	ATCC 6538P	23	0	21	10^{-5}	10^{-1}

Table 3: Antimicrobial activity of methanol extract of *Tectaria coadunate* leaves

S.N.	Bacteria	Reference culture	ZOI Value (mm)			MIC $mg.mL^{-1}$	MBC $mg.mL^{-1}$
			Positive control Ampicillin	Negative Control Methanol	TC leaves extract		
1.	*Escherichia coli*	ATCC 8739	27	0	16	10^{-4}	1
2.	*Klebsiella pneumonia*	ATCC 700603	18	0	9	10^{-2}	10
3.	*Staphylococcus aureus*	ATCC 6538P	23	0	20	10^{-4}	10^{-2}

The high value of ZOI of plant extracts for two gram negative bacteria (*Escherichia coli, Klebsiella pneumonia*) and one gram positive bacteria (*Staphylococcus aureus*) and corresponding values for MIC and MBC shows that the TC

leaves and rhizomes are very potent to prevent the diseases caused by these bacteria.

3.6 Proximate nutritional analysis

The proximate analysis is a set of methods to get information about the nutritional value of food. It uses some chemical-physical properties of a special group of nutrients. Even though, this analysis is not always selective: ammonia is also determined as protein and the nutritional value of the carbohydrate fraction is not determined precisely, we follow it here as it is easy and cheap to conduct the analysis. The results obtained by the proximate analysis of the nutritional value of the fresh and oven dried samples of leaves are summarized in Table 4.It can be ascertained that the TC leaves (young shoots) are very rich in nutritional value and their contents as low percentage fats and high percentage crude fiber stand them in the healthy vegetables category.

Table 4: Percentage composition of nutritional parameters in fresh and oven dried sample of *Tectaria coadunate* leaves

S.N.	Parameters	% in fresh sample	% in oven dried
1.	Moisture	87.23	0
2.	Total ash	1.40	11.04
3.	Crude fat	0.14	1.11
4.	Protein	1.76	13.88
5.	Crude fiber	2.42	19.05
6.	Carbohydrate	6.97	54.93
7.	Vitamin C	-	10.49 mg/100g sample

3.7 GC/MS Analysis

From the mass spectra and chromatograms of methanol extract of TC leaves (Figure 12 and 13) and rhizomes (Figure 14 and 15), the identified major chemical compounds are listed in Table 5 and 6 respectively. The corresponding retention times are also listed.

Table 5: Compounds detected in GC-MS analysis of methanol extract of *Tectaria coadunate* leaves

S.N.	Compounds	Retention time	Percentage area
1.	Octa-9-decyl hexanoate	3.997	0.42%
2.	Ethyl-2-methyl pentanoate	4.112	0.30%
3.	Palmitate <methyl>	4.460	3.93%
4.	Methyl trans-4-methyl cinnamate	4.786	0.56%
5.	Palmitic acid	4.995	23.06%
6.	Dodecyl pentanoate	6.101	0.96%
7.	Methyl linoleate	6.220	0.97%
8.	Phytol	6.449	1.51%
9.	Verbanol<iso>	7.212	1.05%
10.	Neptalic acid	7.173	0.52%

Table 6: Compounds detected in GC-MS analysis of methanol extract of *Tectaria coadunate* rhizomes

S.N.	Compound	Retention time	Percentage area
1.	Methyl stearate	4.459	3.30%
2.	Dodecanoic acid	4.978	3.55%
3.	Decanediol<1,10>	6.261	3.65%
4.	Decylpentanoate	15.375	1.93%
5.	Octanoic acid	16.821	2.26%
6.	Pentadecanoic acid	17.666	2.44%
7.	3-methyl-2-butenyl octanoate	19.655	1.98%
8.	9-octadecanoic acid(z)-trimethyl silyl ester	24.492	0.70%
9.	Dihydroagarofuran<cis>	24.704	1.05%

As shown in Table 5, the palmitic acid with percentage area 23.06% appears as the most abundant chemical compound in TC leaves. It is a common saturated fatty acid and is a major body component of humans. It makes up 21–30% (molar) of human depot fat [17] and is a major, but highly variable, lipid component of human breast milk [18]. It adds an extra advantage to the humans consuming TC leaves in their balanced diet. Similar analysis for the TC rhizomes revealed that Decendiol<1, 10>, Dodecanoic acid (Lauric acid) and Methyl stearate exist as major chemical compounds with percentage area of 3.65%, 3.55% and 3.30% respectively, as listed in Table 6. As the first compound" Decendiol<1, 10>" has been using as an antiasthmatics, bronchodilators and drugs for the disorder of the urinary system, second compound "Dodecanoic acid" is believed to have antimicrobial properties and the third compound "Methyl stearate" as an antifoaming agent and fermentation nutrient, the TC rhizomes could be a potential prototype drug in pharmacology and pharmaceutics and can be used to derive the effective drugs having different specific benefits.

Fig 1: The young shoots emerging from ground: the fiddleheads are covered with fuzz and need to be cleaned before cooking

Fig 2: The young shoots of edible fern (*Tectaria coadunate, kali niyuro*, कालिन्युरो): become ready to cook.

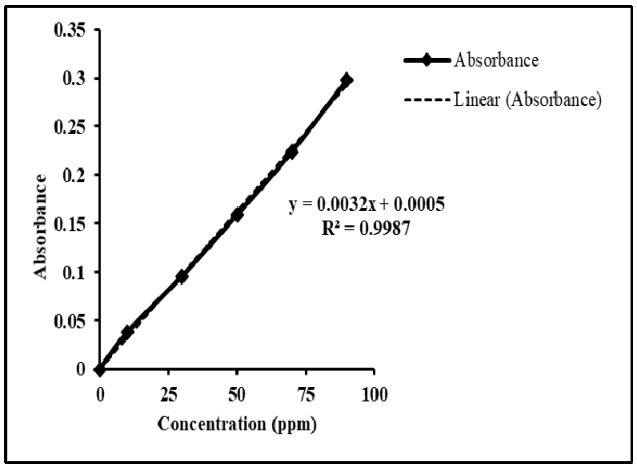

Fig 3: Calibration curve for Gallic acid

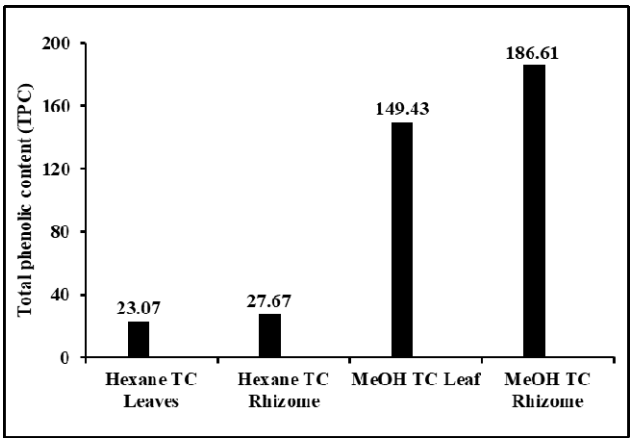

Fig 4: Total phenolic content of different extracts. TC stands for *Tectaria coadunate*.

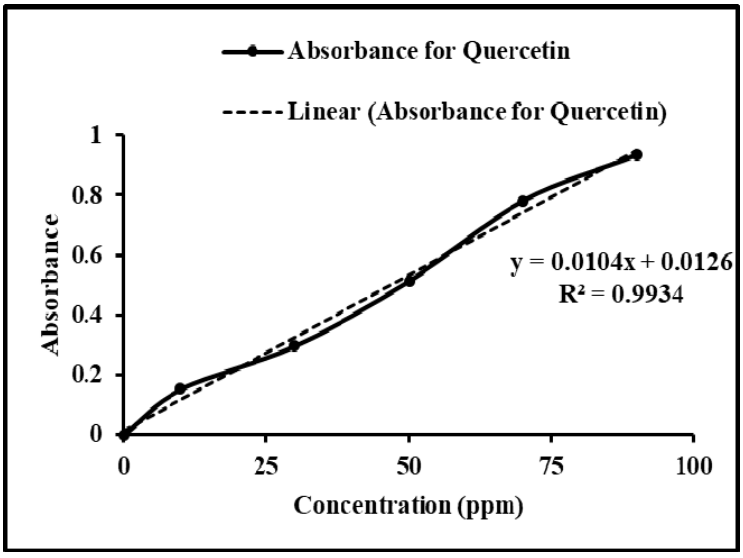

Fig 5: Calibration curve for Quercetin

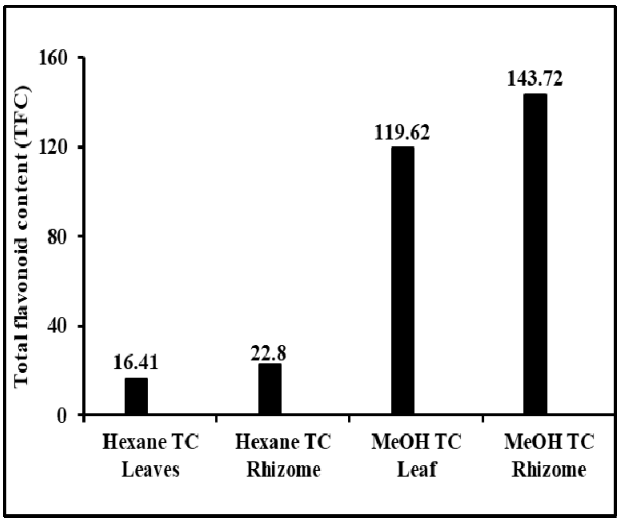

Fig 6: Total flavonoid content of different extracts.TC stands for *Tectaria coadunate*

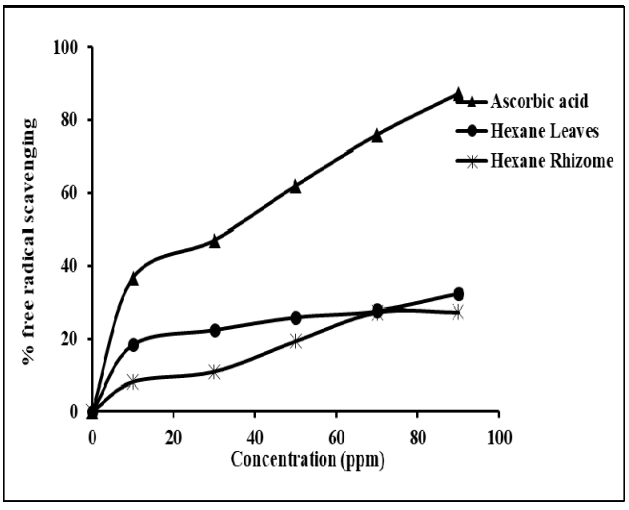

Fig 7: Comparison of antioxidant activity of hexane extracts with ascorbic acid

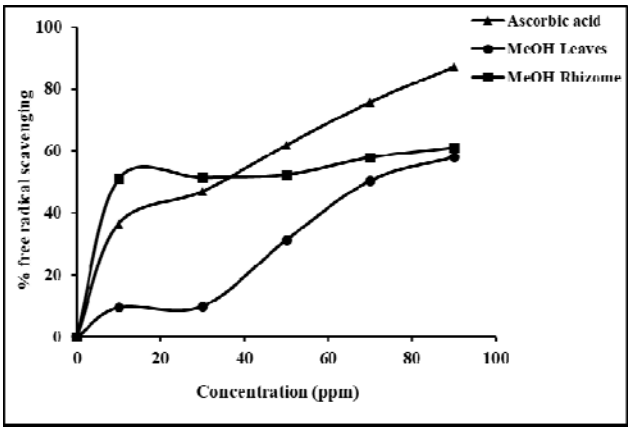

Fig 8: Comparison of antioxidant activity of methanol (MeOH) extracts with ascorbic acid

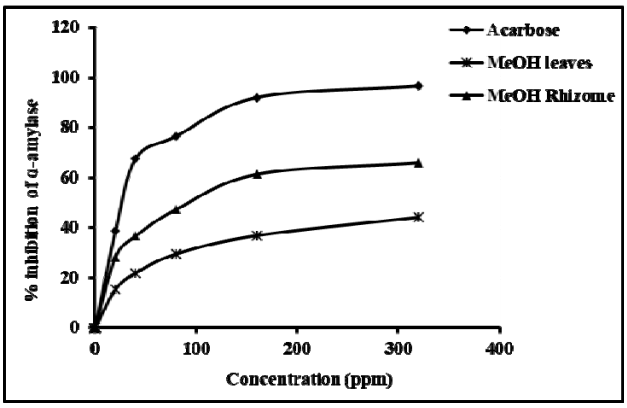

Fig 10: Comparison of Antioxidant activity of acarbose and methanol (MeOH) extracts of leaves and rhizome

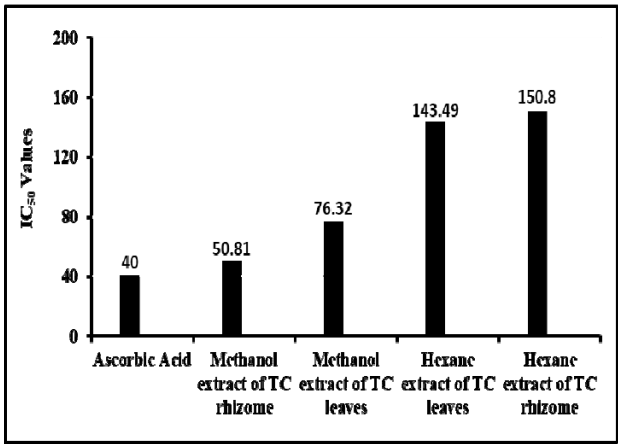

Fig 9: IC_{50} values for ascorbic acid, methanol and hexane extracts of TC leaves and rhizome. TC stands for *Tectaria coadunate*.

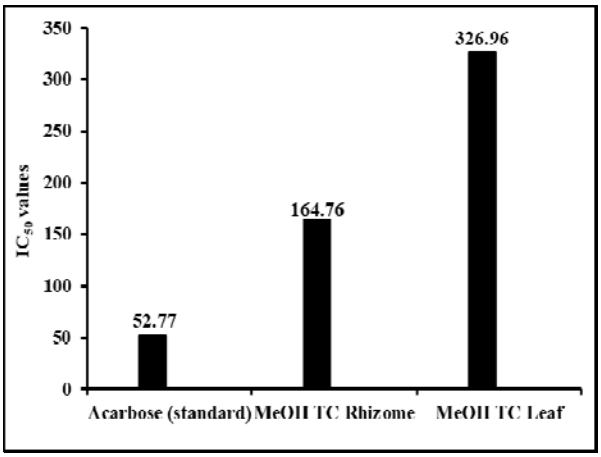

Fig 11: IC_{50} values for acarbose and methanol extracts of TC leaf and rhizome. TC stands for *Tectaria coadunate*

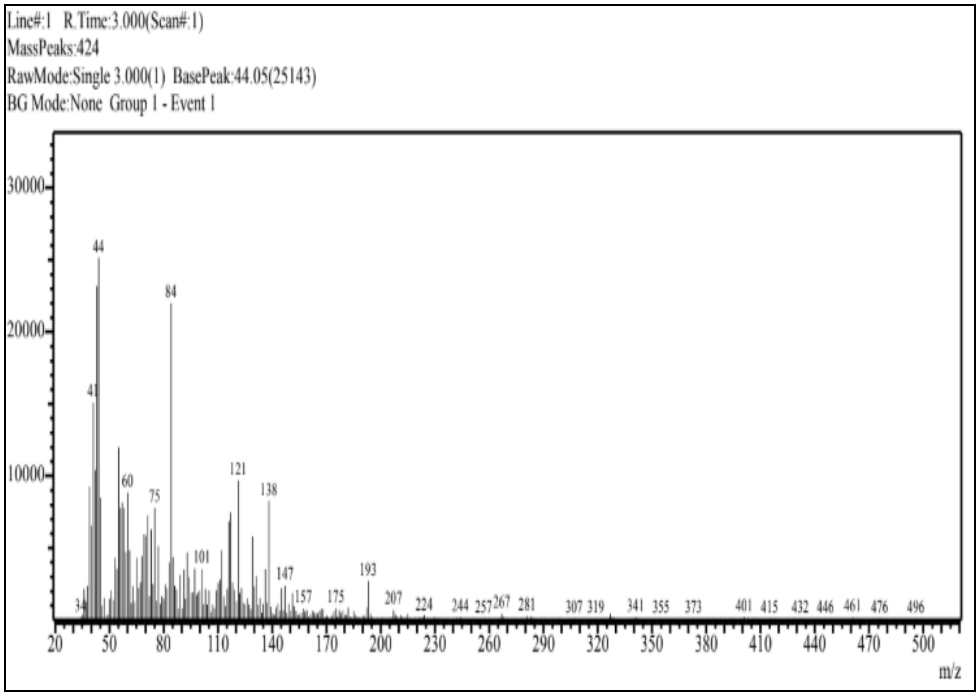

Fig 12: Mass Spectrum of methanol extract of *Tectaria coadunate* leaves

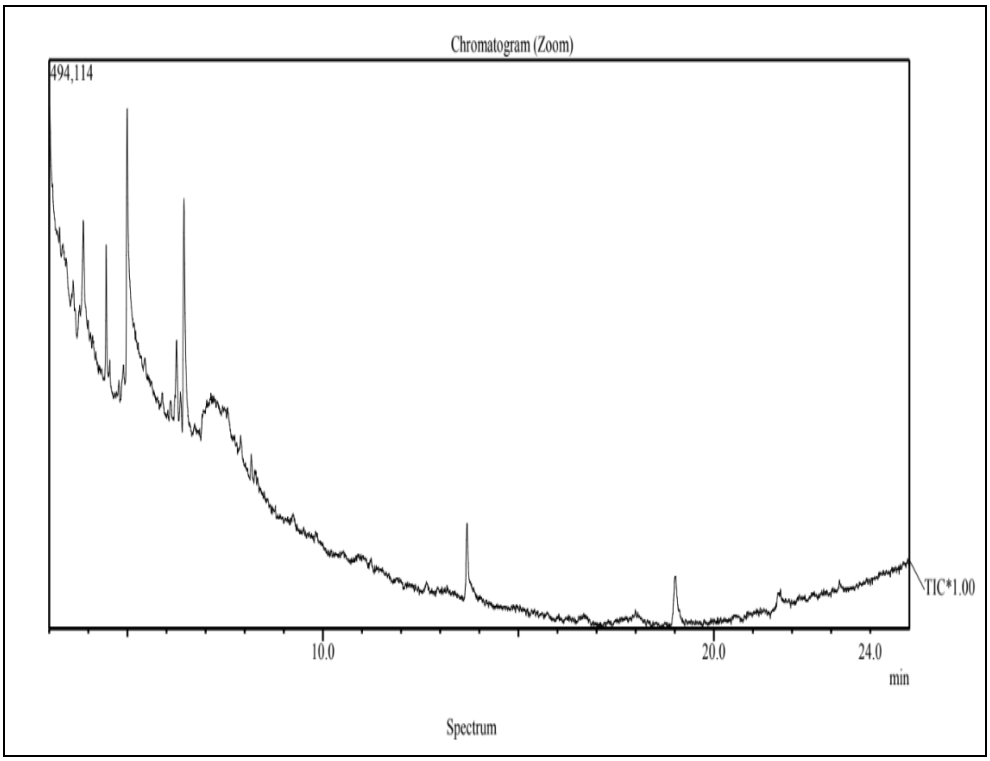

Fig 13: Gas Chromatogram of methanol extract of *Tectaria coadunate* leaves

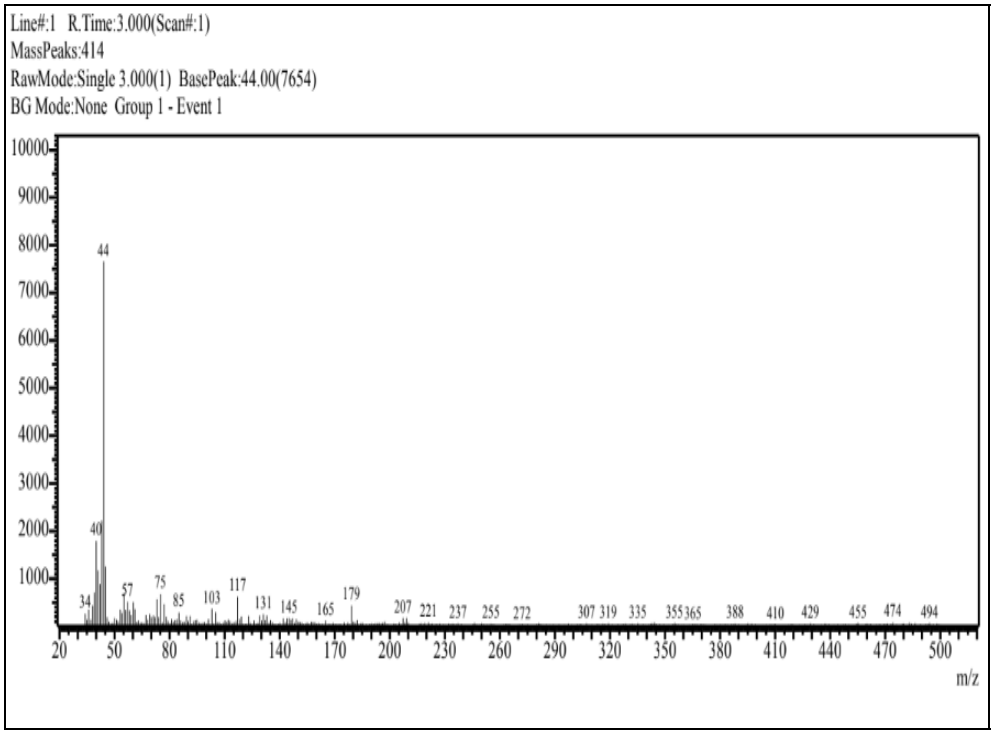

Fig 14: Mass Spectrum of methanol extract of *Tectaria coadunate* rhizomes

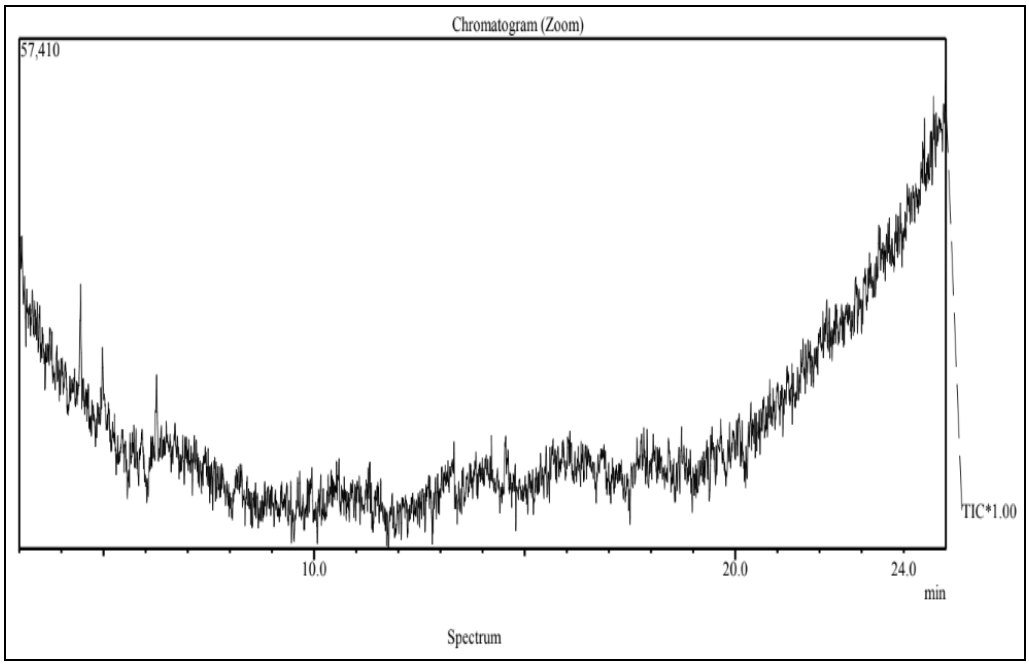

Fig 15: Gas Chromatogram of methanol extract of *Tectaria coadunate* rhizomes

4. Conclusion

The phytochemical screening of methanol and hexane extracts of *Tectaria coadunate* (kali niyuro, कालिन्युरो in Nepalese language) revealed the presence of polyphenols, terpenoids, saponins, tannins, alkaloids, quinones, glycosides, steroids, phenolic compounds and flavonoids. The methanol extract of TC rhizomes is found to be the most potent natural antioxidant among all extracts because of having IC_{50}value as comparable as the standard. Furthermore, both methanol extracts of leaves and rhizomes are found to contain higher amount of phenolic and flavonoid content as compared to the corresponding hexane extracts whereas among leaves and rhizomes, total phenolic content (TPC) and total flavonoid content (TFC) are estimated to be higher in rhizome extracts.

The leaves extract having very high IC_{50} value shows little antidiabetic activity whereas rhizome extract has IC_{50} value sufficient enough to show significant antidiabetic activity. The percentage composition of different nutrition parameters (moisture, total ash, crude fat, protein, crude fiber, carbohydrate) are found to be 87.23, 1.40, 0.14, 1.76, 2.42, and 6.97% in fresh leaves (young edible shoots) which make them good alternative to the hygienic vegetables. Significantly less value of fat but high value of crude fiber makes the leaves and young shoots healthy food and can be recommended them to hypertension patient for regular consumption. The high ZOI values of *Tectaria coadunate* extracts (leaves and rhizome) for *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus* and corresponding MIC and MBC values declare their potentialities against all diseases caused by these bacteria.

From the GC-MS analysis of *Tectaria coadunate* extracts, Palmitic acid has been found to be the most abundant chemical compound in the leaves and Decenediol<1,10>, Dodecanoic acid and Methyl stearate are in the rhizomes. The Palmitic acid being a major body component of humans that makes up 21–30% (molar) of human depot fat and a major lipid component of human breast milk, its presence in the leaves (and young edible shoots) makes *Tectaria coadunate* best alternative hygienic vegetable. Furthermore, the major chemical compounds found in the rhizomes are already in use in pharmacology and pharmaceutics as the y possess antimicrobial properties. Due to this, the *Tectaria coadunate* rhizomes could be a future potential prototype drug.

Even though it was desired to prepare the plant extracts in other major solvents to reveal more phytochemicals; to assess antiallergic, antihelminthic, anticancer, antiseptic and anti-inflammatory tests, due to lack of enough budget and time factor, we studied *in vitro* test only. *In vivo* test can be carried out to further galvanize the results obtained here. Similarly, in nutritional analysis, to make proximate nutritional parameters more precise, qualitative and quantitative analysis of minerals and estimation of calorific value can be studied to assess other major vitamins.

5. Acknowledgement

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